*Indian Journal of Agricultural Sciences* **96** (2): 224–229, February 2026/Article
https://doi.org/10.56093/ijas.v96i2.171478

# An improved DNA extraction method and standardisation of transmission studies of enation leaf curl virus in okra (*Abelmoschus esculentus*)

NISHANT[1], SUSHEEL KUMAR SHARMA[1], DAMINI DIKSHA[1], RESHAV NAIK[1], NIRANKAR[1] and RAMESH KUMAR YADAV[1]*

*ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India*

Received: 04 September 2025; Accepted: 19 January 2026

ABSTRACT

In India, okra (*Abelmoschus esculentus* L.) is severely affected by OELCuV (Okra enation leaf curl virus) which is influenced by vector (white fly, *Bemisia tabaci*), host and environment. DNA isolation from Malvaceae plant families is difficult due to high mucilage content, which binds to secondary metabolites and interferes with molecular studies. The study was carried out during 2023–2024 at ICAR-Indian Agricultural Research Institute, New Delhi to utilise a supplemented extraction buffer with 2.0 g of polyvinylpyrrolidone (PVP) per 50 mL to neutralise polyphenols. The samples were treated with chloroform: isoamyl alcohol (24:1) twice for effective phase separation and mucilage removal. Additionally, a washing step with 10 mM ammonium acetate was incorporated to eliminate residual polysaccharides and proteins, enhancing DNA purity. This protocol yielded high quality DNA with an average concentration of 1501.35 ng/µL and purity ratios of A260/A280 = 1.82 and A260/A230 = 1.78, outperforming commercial kits and conventional methods of DNA isolation. Using a controlled whitefly culture, the study assessed *Begomovirus abelmoschusenation* transmission efficiency across various okra seedling growth stages. Virus acquisition and inoculation required minimum access periods of 45 min, with transmission efficiency increasing over time and vector density. Maximum transmission (100%) was observed at 24 h after acquisition and inoculation access periods using 12 whiteflies/seedling. Younger seedlings (10–12 days old) exhibited the highest susceptibility, while no transmission occurred in plants older than 45 days. Furthermore, PCR analysis confirmed the absence of seed transmission. The study offered key insights into *Begomovirus abelmoschusenation* epidemiology and improved okra viral disease diagnostics and management.

**Keywords**: *Begomovirus abelmoschusenation*, DNA extraction protocol, Okra enation leaf curl virus, Seed transmission

Okra (*Abelmoschus esculentus* L.), a warm season crop from the Malvaceae family, is amphidiploid (2n = 82–130) (Durazzo *et al.* 2019). India leads global production, contributing over six million tonnes annually with 544 thousand hectares and 12.28 t/ha productivity. India produces 6,889 thousand tonnes annually, led by Gujarat, West Bengal, Bihar and Madhya Pradesh (APEDA 2023, Statista 2023). The Geminiviridae family includes four different genera i.e. *Mastrevirus, Topocuvirus, Curtovirus* and *Begomovirus*, the largest genus with 322 recognised species (Fiallo-Olive *et al.* 2021). The virus genome consists of DNA-A and DNA-B molecules, while satellite particles assist in replication and transmission (Brown *et al.* 2015). Okra yield is significantly reduced by many viral diseases, with okra enation leaf curl virus (OELCuV) causing severe losses in India (Singh *et al.* 1986). Symptoms include enations on leaves, leaf curling, stunting and deformed fruits. OELCuV caused by okra enation leaf curl virus (*Begomovirus abelmoschusenation*) from the *Begomovirus* genus (Lazarowitz 1992). PCR (Polymerase chain reaction) and sequencing offer reliable virus detection (Kushwaha *et al.* 2010), but DNA extraction from mucilaginous plants like okra remains challenging because mucilage or secondary metabolites hinder isolation (Ghosh *et al.* 2009). Mucilage contamination inhibits Taq polymerase and affects enzymatic reactions (Bayer *et al.* 1999). Since Doyle and Doyle (1987), DNA extraction optimisation has focused on overcoming challenges posed by polysaccharide and phenolic compound precipitation. The standard CTAB (Cetyltrimethylammonium bromide) method often leads to contamination, requiring time-consuming purification steps, reducing DNA yield and PCR reproducibility. The whitefly, *Bemisia tabaci,* is the major vector to spread this begomovirus, with key factors like host plant age and seed transmission influencing transmission dynamics. This study established a whitefly culture to assess OELCuV transmission by *Bemisia tabaci*, to evaluate okra plant susceptibility at various growth stages, to explore

[1]ICAR-Indian Agricultural Research Institute, New Delhi.
*Corresponding author email: rkyadavneh@gmail.com

seed transmission, validate the DNA extraction protocol and screen different okra germplasm to understand the virus-host relationship.

## MATERIALS AND METHODS

*Methods of DNA extraction and validation*: Total DNA was extracted employing 10 different protocols, including modified methods introduced in this study, viz. CTAB method (Doyle and Doyle 1987), DNeasy Plant Mini Kit (QIAGEN, Germany), Genomic DNA Purification Kit (Thermo Scientific™), Improved method for mucilaginous plants (Ghosh *et al.* 2009), Viral DNA isolation in okra (Jeyaseelan *et al.* 2019), DNA isolation for MAPs (Pirttila *et al.* 2001), Modified CTAB protocol (Allen *et al.* 2006), Okra DNA from mucilage-rich tissues (Adiger and Sridevi 2014), and DNA isolation from *Abelmoschus* spp. (Seth *et al.* 2018). Samples collected from different regions (Supplementary Table 1) of states in India showing okra enation leaf curl symptoms (Fig.1) were validated using a modified DNA extraction protocol. The extracted DNA (by modified protocol) from 30 samples collected from major hotspot region of India were further screened using OELCuV-specific primers, with the forward primer sequence 5′-CGCTATAAGTACTTGCGCACTAAG-3′ and the reverse primer sequence 5′-CATTCGTGATTTTGTGACGCGG-3′ (Naresh *et al.* 2019). PCR amplification with OELCuV-specific primers began with an initial denaturation step at 94°C for 4 min, followed by 35 cycles consisting of denaturation at 94°C for 45 sec, primer annealing at 58°C for 45 sec, and DNA extension at 72°C for 1 min, concluding with a final extension step at 72°C for 10 min. Protocols 1–9 were followed as per the authors' descriptions or the manufacturer's guidelines. Each extraction protocol was tested twice.

### *Modified DNA extraction protocol*

*Preparation of plant tissues:* Plant tissues (leaves and seedlings) were rinsed with autoclaved distilled water, dried with sterile blotting paper, and 80–100 mg finely powdered in liquid nitrogen.

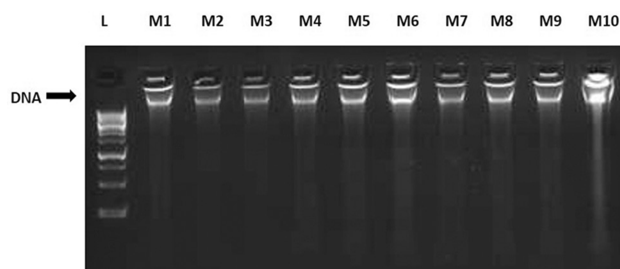


Fig. 2 Agarose gel showing okra genomic DNA from different protocols.
L, 1 kb ladder; M1, Doyle and Doyle (1987); M2, QIAGEN; M3, Thermo; M4, Ghosh *et al.* (2009); M5, Jeyaseelan *et al.* (2019); M6, Pirttila *et al.* (2001); M7, Allen *et al.* (2006); M8, Adiger and Sridevi (2014); M9, Seth *et al.* (2018); M10, Modified method used in the study.

*Cell lysis*: The powdered tissue was added to 2 mL of freshly prepared CTAB extraction buffer and incubated at 65°C for 1.5 h with intermittent mixing. The composition of 20 mL of CTAB buffer was as follows: 2.0 mL of 1 M Tris (*p*H 8.0), 800 µL of 0.5 M EDTA (*p*H 8.0), 5.6 mL of 5 M NaCl, 7 mL of 10% CTAB, 40 µL of β-mercaptoethanol, 800 mg of PVP and 4.56 mL of sterile distilled water (SDW).

*Chloroform:isoamyl alcohol extraction (First step)*: An equal volume of chloroform:isoamyl alcohol (24:1) was added, mixed and centrifuged at 12,000 rpm for 10 min, then the supernatant was collected.

*Chloroform:isoamyl alcohol extraction (Second step)*: The supernatant was re-extracted with an equal volume of chloroform:isoamyl alcohol (24:1), mixed, centrifuged at 12,000 rpm for 10 min and the resulting supernatant collected for further processing.

*DNA precipitation*: Chilled isopropanol was added to the 0.8 volume of supernatant and the mixture was incubated overnight at 4°C.

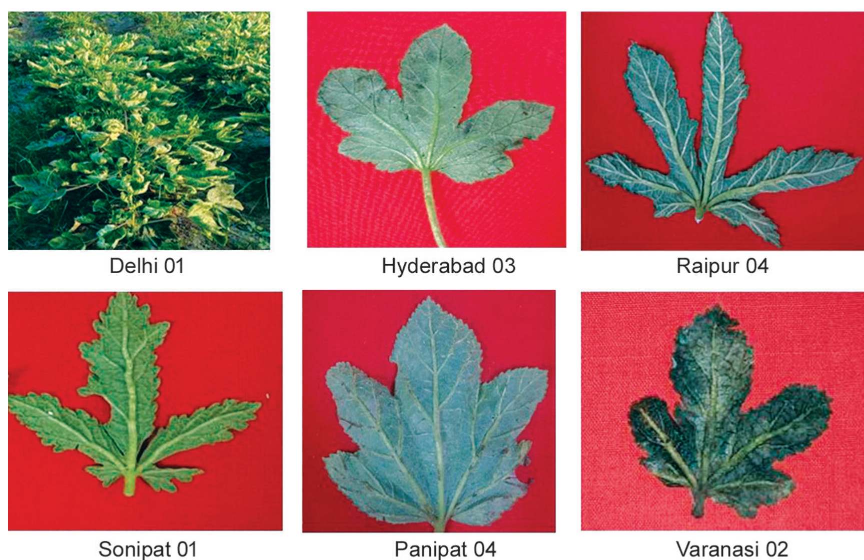


Fig. 1 Infected okra plants and leaves samples from major Indian hotspot showing symptoms of okra enation leaf curl virus.

*Centrifugation*: The mixture was centrifuged at 12,000 rpm for 10 min to collect the DNA precipitate as pellets, followed by the addition of 50–100 µL of water to the precipitated tube.

*RNA removal*: The DNA pellet was treated with RNase A (3–4 µL/100 µL of DNA solution) and incubated at 37°C for 1 h.

*Ethanol and ammonium acetate washes*: The pellet was washed sequentially with 500 µL of 100% ethanol and for next washing, 500 µL of 70% ethanol and 500 µL of 10 mM ammonium acetate were used.

*Drying and dissolution*: The DNA pellet was air-dried and dissolved in 100 µL double-distilled water.

*DNA assessment*: DNA

concentration was measured by loading 1.0 µL of each sample on to a Nanodrop 2000 (Thermo Scientific) at 260 nm wave length, and quality was assessed using A260/A280 and A260/A230 ratios.

*Insect transmission*: Whitefly culture was established by collecting adults from okra (DOV-92), rearing them on eggplants (Pusa Bhairav) under controlled conditions and maintaining a virus-free stock in 40-mesh nylon-covered wooden cages in a glasshouse. OELCuV-infected samples were collected in 2023 from the Vegetable Research Farm and pure virus isolates (OELCuV) were maintained via whitefly inoculation at the Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi (28°38´N, 77°09´E; at an elevation of 228.61 m amsl). For transmission threshold studies, non-viruliferous *Bemisia tabaci* were allowed a 24 h acquisition access period (AAP) on infected plants (DOV-8063) and released (0–25/plant) onto 12-day-old okra seedlings for a 24 h inoculation access period (IAP), followed by vector control with spiromesifen 240 SC. To assess the effect of host plant age on susceptibility, 12 viruliferous whiteflies were confined on plants aged 10–45 days, infection data were used to calculate the single whitefly transmission rate using Gibbs and Gower's (1960) formula:

$$C^* = 1 - (1 - T\%)\ \hat{}\ 1/W^*$$

Where C*, Transmission rate for a single whitefly; T, Transmission rate (T = A/B); A, No. of infected plants; B, No. of inoculated plants; W, No. of whiteflies/inoculated plant.

For virus acquisition studies, ≤ 24 h-old aviruliferous whiteflies were allowed to feed on symptomatic okra, petioles placed in 0.9% agar in insect breeding dishes, then transferred to virus-free leaves and adults were collected at specific intervals (0–24 h).

*Seed transmission*: Matured seeds from symptomatic (DOV-8063) and non-symptomatic (DOV-92) okra enation leaf curl virus plants were harvested, washed with 2% sodium hypochlorite for 2 min and rinsed with sterile distilled water. Three sets of 25 seeds each from healthy and diseased plants were sown in separate pots (Venkataravanappa *et al.* 2012). Germination was recorded and pots were kept in a glasshouse for one month for symptom development. Seedlings were sprayed with spiromesifen 240 SC (22.9% w/w) every 10 days to prevent insect transmission. The presence of the virus in the seedlings was confirmed by PCR using OELCuV-specific primers (forward: 5′-CGCTATAAGTACTTGCGCACTAAG-3′; reverse: 5′-CATTCGTGATTTTGTGACGCGG-3′) (Naresh *et al.* 2019).

## RESULTS AND DISCUSSION

During the extraction process, a notable reduction in DNA yield and quality was observed, likely due to the precipitation of polysaccharides with DNA and irreversible binding of oxidised polyphenols to nucleic acids. A modified CTAB method incorporating 2.0 g PVP/50 mL in the extraction buffer effectively mitigates mucilage and polyphenols by forming hydrogen-bonded complexes. PVP enhances phenol removal and prevents oxidation, yielding higher DNA concentrations than sorbitol or SDS. PVP-10 (M.W. 10,000) is preferred for better yields with minimal coprecipitates. Fresh solutions are recommended, often requiring heat to dissolve (Kistler 2012).

This standardised protocol was developed in which polyvinylpyrrolidone (PVP; 2.0 g/50 mL) was incorporated to neutralise polyphenols. The samples were extracted twice with chloroform:isoamyl alcohol (24:1) to achieve efficient phase separation and removal of mucilage. Furthermore, an additional washing step with 10 mM ammonium acetate was introduced to eliminate residual polysaccharides and proteins, thereby enhancing DNA purity and stability. With this method, high-quality DNA was obtained with an average concentration of 1501.35 ng/µL and purity ratios (A260/A280: 1.82, A260/A230: 1.78). In contrast, other methods yielded moderate DNA concentrations (519.6–1015.6 ng/µL). The inclusion of 10 mM ammonium acetate during washing improved DNA purity by removing carbohydrates and proteins while stabilising the DNA (Boom *et al.* 1990). The use of chloroform:isoamyl alcohol twice effectively removed mucilage, with chloroform aiding phase separation and isoamyl alcohol reducing foaming or phosgene formation (Sambrook and Russell 2001, Green and Sambrook 2012). QIAGEN and Thermo Scientific™ kits

Table 1 Spectrophotometer reading for the quantity and quality of total DNA extracted from leaf samples of okra

| Method | Concentration (ng/µL) | Ratio (A260/A280) | Ratio (A260/A230) | Quality of DNA |
|---|---|---|---|---|
| Doyle and Doyle (1987) | 339.5–353.3 | 1.8 | 0.92–1.00 | + |
| QIAGEN, Germany | 4.9–52.1 | 1.37–1.77 | 0.52–2.64 | ** |
| Thermo Scientific™ | 1.5–14.1 | 2.03–3.20 | 0.83–2.99 | ** |
| Ghosh *et al.* (2009) | 107.8–443.8 | 1.56–2.11 | 0.48–1.31 | + |
| Jeyaseelan *et al.* (2019) | 151.6–284.7 | 1.64–2.02 | 1.61–1.85 | + |
| Pirttila *et al.* (2001) | 519.6–1015.6 | 1.70–1.90 | 1.56–1.71 | ++ |
| Allen *et al.* (2006) | 205.6–384.7 | 1.56–1.96 | 1.89–2.02 | + |
| Adiger and Sridevi (2014) | 1073.6–1102.3 | 1.42–1.68 | 1.26–130 | + |
| Seth *et al.* (2018) | 506.2–724.8 | 1.96–2.00 | 1.26–1.83 | + |
| Modified protocol | 1370–1632.7 | 1.78–1.86 | 1.59–1.99 | +++ |

**, Very poor-quality DNA; +, Bad quality DNA, ++; Good quality DNA; +++, Excellent DNA quality.

yielded DNA concentrations ranging from 1.5–52.1 ng/µL, with purity ratios (A260/A280: 1.37–3.20, A260/A230: 0.52–2.99) indicating poor DNA quality. Low yields were due to mucilage-induced column clogging. Furthermore, NanoDrop analysis revealed that the quality metrics for most DNA samples from these different protocols fell outside the acceptable ranges (Table 1). These inhibitory interactions have similarly been reported in previous studies involving DNA isolation from Malvaceae (Aljanabi *et al.* 1999). Fang *et al.* (1992) also emphasised the difficulty in handling DNA due to viscous polysaccharides, which hinder pipetting and amplification.

Modified protocols (Pirttila *et al.* 2001, Ghosh *et al.* 2009, Adiger and Sridevi 2014) produced brighter bands, while commercial kits and other methods showed faint bands. Genomic DNA was successfully extracted from okra leaves exhibiting enation leaf curl symptoms using various modified protocols. Among these, the modified method produced the most intense bands on agarose gel electrophoresis (Supplementary Fig. 1), indicating superior DNA yield and purity. To assess the robustness of the optimised extraction protocol, it was further validated using 45 field-collected okra leaf infected samples (Supplementary Table 1) from major hotspot regions across India (Fig. 1). DNA was successfully isolated from all samples (Lanes 1–45), with clear and consistent high-molecular weight bands, demonstrating the method's reliability for large-scale field samples processing (Supplementary Fig. 1). Subsequently, 30 samples exhibiting strong band intensity were selected for PCR-based detection of OELCuV. All selected samples tested positive, yielding the expected amplicon size of 327 bp (Supplementary Fig. 2), thereby confirming the presence of the virus and the suitability of the extracted DNA for downstream molecular applications.

Whitefly transmission characteristics of okra enation leaf curl virus were consistent with those of other begomoviruses and the established transmission mechanisms of geminiviruses. Studies on OELCuV and its vector, *Bemisia tabaci*, revealed that virus transmission did not occur within the first 30 min of the acquisition access period (AAP). A minimum of 45 min of AAP is required for virus acquisition, resulting in a 10% transmission rate. Transmission efficiency increases significantly over time, with 100% transmission observed after a 24 h AAP (Table 2). Similarly, a minimum inoculation access period (IAP) of 45 min was necessary for *Bemisia tabaci* to transmit OELCuV, achieving 100% transmission after 24 h of innoculation access period (IAP). The findings demonstrated a strong positive correlation between the duration of AAP/IAP and transmission efficiency. Transmission rate (T) and estimated transmission rate for a single whitefly (C*) increased proportionally with the number of whiteflies/plant, reaching their highest values at a 24 h AAP and IAP (Table 2). The study revealed that two *Bemisia tabaci* individuals could achieve a 10% transmission rate of OELCuV, while 12 whiteflies/seedling were necessary to attain 100% transmission efficiency. Transmission percentage increased proportionally with the number of

Table 2 Evaluation of minimum acquisition and inoculation access periods for whitefly-mediated OELCuV transmission in okra

| Determination of minimum AAP | | | | | Determination of minimum IAP | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AAP | IAP | Transmission | | | AAP | IAP | Transmission | | |
| | | Plants infected (A)/inoculated (B) | % of plants infected (T=A/B) | Estimated transmission rate of single whitefly (C*) | | | Plants infected (A)/inoculated (B) | % of plants infected (T=A/B) | Estimated transmission rate of single whitefly (C*) |
| 0 min | 12 h | 0/10 | 0 | 0 | 12 h | 0 min | 0/10 | 0 | 0 |
| 5 min | | 0/10 | 0 | 0 | | 5 min | 0/10 | 0 | 0 |
| 10 min | | 0/10 | 0 | 0 | | 10 min | 0/10 | 0 | 0 |
| 15 min | | 0/10 | 0 | 0 | | 15 min | 0/10 | 0 | 0 |
| 20 min | | 0/10 | 0 | 0 | | 20 min | 0/10 | 0 | 0 |
| 25 min | | 0/10 | 0 | 0 | | 25 min | 0/10 | 0 | 0 |
| 30 min | | 0/10 | 0 | 0 | | 30 min | 0/10 | 0 | 0 |
| 45 min | | 1/10 | 10 | 0.92 | | 45 min | 2/10 | 20 | 0.93 |
| 1 h | | 2/10 | 20 | 0.93 | | 1 h | 3/10 | 30 | 0.94 |
| 4 h | | 3/10 | 30 | 0.94 | | 4 h | 4/10 | 40 | 0.95 |
| 8 h | | 5/10 | 50 | 0.95 | | 8 h | 7/10 | 70 | 0.97 |
| 12 h | | 7/10 | 70 | 0.97 | | 12 h | 8/10 | 80 | 0.98 |
| 16 h | | 8/10 | 80 | 0.98 | | 16 h | 9/10 | 90 | 0.99 |
| 24 h | | 10/10 | 100 | 1.00 | | 24 h | 10/10 | 100 | 1.00 |

OELCuV, Okra enation leaf curl virus; AAP, Acquisition access period; IAP, Inoculation access period. In each case, 12 whiteflies/plant were used.

Table 3 Effect of insect quantity on OELCuV transmission and incubation period after 24 h of virus acquisition and inoculation feeding

| Number of whiteflies/ plant (W) | No. of plants infected (A)/ noculated (B) | Transmission rate (T= A/B) (%) | Estimated transmission rate of single whitefly (C*) |
|---|---|---|---|
| 0 | 0/10 | 0 | 0 |
| 1 | 0/10 | 0 | 0 |
| 2 | 1/10 | 10 | 0.5 |
| 3 | 2/10 | 20 | 0.7 |
| 5 | 4/10 | 40 | 0.8 |
| 7 | 6/10 | 60 | 0.9 |
| 10 | 8/10 | 80 | 0.9 |
| 12 | 10/10 | 100 | 1.0 |
| 15 | 10/10 | 100 | 1.0 |
| 20 | 10/10 | 100 | 1.0 |
| 25 | 10/10 | 100 | 1.0 |

OELCuV, Okra enation leaf curl virus.

Table 4 Effect of seedling age (DOV-8063) on OELCuV transmission by *Bemisia tabaci*

| Age of the seedlings | Number of whiteflies (W) | No. of plants infected (A)/ inoculated (B) | Trans-mission (T=A/B) | Estimated transmis-sion rate of single whitefly (C*) |
|---|---|---|---|---|
| 10 days | 12 | 10/10 | 1.00 | 1.00 |
| 15 days | 12 | 10/10 | 1.00 | 1.00 |
| 20 days | 12 | 6/10 | 0.6 | 0.96 |
| 25 days | 12 | 3/10 | 0.3 | 0.94 |
| 30 days | 12 | 2/10 | 0.2 | 0.93 |
| 35 days | 12 | 1/10 | 0.1 | 0.92 |
| 45 days | 12 | 0/10 | 00 | 0.91 |

OELCuV, Okra enation leaf curl virus.

whiteflies/seedling (Table 3). The highest transmission rate (100%) occurred in 10-day-old okra seedlings, whereas the lowest rate (10%) was observed in 35-day-old seedlings. OELCuV transmission included a latent period during which the virus travels from the insect's gut to its salivary glands. Virus acquisition, requiring access to specific phloem cells, is slower compared to transmission, which occurs during salivation. This process involves numerous probing attempts by the insect, with relatively few resulting in successful virus acquisition (Stafford *et al.* 2012). The study confirmed that all tested okra seedling ages were susceptible to OELCuV, but susceptibility decreased with age. No transmission occurred in plants older than 45 days. Transmission efficiency was highest (T and C* = 1.00) in 12-day-old seedlings and dropped to 30% in 25-day-old plants (Table 4). Plants exposed to non-viruliferous whiteflies showed no symptoms and PCR tests detected no virus. Additionally, none of the 50 seedlings grown from seeds of infected plants showed symptoms or no virus presence was confirmed in PCR using OELCuV-specific primers (Naresh *et al.* 2019), confirming that OELCuV is not seed-transmitted, consistent with earlier findings on geminiviruses (Brown *et al.* 2012). OELCuV transmission efficiency increased with more whiteflies and longer AAP/IAP but decreased with seedling age (Muniyappa *et al.* 2000).

This study developed an optimised DNA extraction protocol for okra using PVP-supplemented CTAB buffer, ammonium acetate washing and repeated chloroform: isoamyl alcohol purification, effectively overcoming mucilage and polyphenol interference. The method consistently yielded high-quality genomic DNA and outperformed commercial kits or traditional protocols. Evaluation using 45 field-collected samples consistently generated strong DNA bands and enabled accurate PCR-based detection of OELCuV. Transmission assays with *Bemisia tabaci* revealed that the virus requires at least 45 min for acquisition and inoculation, with transmission efficiency rising with longer access periods and increased vector numbers. Younger seedlings (10–12 days) were most susceptible, while plants older than 45 days showed no transmission. PCR analysis showed no evidence of seed-mediated transmission of the virus. These findings strengthened diagnostic reliability, enhanced epidemiological understanding and devising the management strategies of OELCuV in okra.

## REFERENCES

Adiger S and Sridevi O. 2014. Isolation of DNA from mucilage-rich okra (*Abelmoschus esculentus* L.) for PCR analysis. *Indian Journal of Animal Nutrition* **7**(16): 2306–09.

Ahmed N, Nawaz S, Iqbal A, Mubin M, Butt A, Lightfoot D A and Maekawa M. 2013. Extraction of high-quality intact DNA from okra leaves despite their high content of mucilaginous acidic polysaccharides. *Bioscience Methods* **4**(4): 19–22.

Aljanabi S M, Forget L and Dookun A. 1999. An improved and rapid protocol for the isolation of polysaccharide and polyphenol-free sugarcane DNA. *Plant Molecular Biology Reporter* **17**: 8–13.

Allen G C, Flores-Vergara M A, Krasynanski S, Kumar S and Thompson W F. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols* **1**(5): 2320–25.

APEDA. 2023. The Agricultural and Processed Food Products Export Development Authority 2021. AgriExchange. https://agriexchange.apeda.gov.in

Bayer C, Fay M F, De Bruijn A Y, Savolainen V, Morton C M, Kubitzki K, Alverson W S and Chase M W. 1999. Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: A combined analysis of plastid ATP B and rbc L DNA sequences. *Botanical Journal of the Linnean Society* **129**(4): 267–303.

Boom R C J A, Sol C J, Salimans M M, Jansen C L, Wertheim-van Dillen P M and Van der Noordaa J P M E. 1990. Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology* **28**(3): 495–503.

Brown J K, Fauquet C M, Briddon R W, Zerbini M, Moriones E and Navas-Castillo J. 2012. Geminiviridae. *(In) Virus Taxonomy: Classification and Nomenclature of Viruses, Ninth Report of the International Committee on Taxonomy of Viruses,* pp. 351–73. King A M Q, Adams M J, Carstens E B and Lefkowitz E J (Eds). San Diego: Associated Press, Elsevier Inc.

Brown J K, Zerbin F M, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva J C F, Fiallo-Olive E, Briddon R W, Hernandez-Zepeda C, Idris A, Malathi V G, Martin D P, Rivera-Bustamante R, Ueda S and Varsani A. 2015. Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Advances in Virology* **160**(6): 1593–1619.

Doyle J J and Doyle J L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**(1): 11–15.

Durazzo A, Lucarini M, Novellino E, Souto E B, Daliu P and Santini A. 2019. *Abelmoschus esculentus* (L.): Bioactive components' beneficial properties focused on anti-diabetic role for sustainable health applications. *Molecules* **24**(1): 38.

Fang G, Hammar S and Grumet R. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biofeedback* **13**: 52–53.

Fiallo-Olive E, Lett J M, Martin D P, Roumagnac P, Varsani A, Zerbini F M and Navas-Castillo J. 2021. ICTV virus taxonomy profile: Geminiviridae 2021. *Journal of General Virology* **102**(12): 001696.

Ghosh R, Paul S, Ghosh S K and Roy A. 2009. An improved method of DNA isolation suitable for PCR-based detection of Begomoviruses from jute and other mucilaginous plants. *Journal of Virological Methods* **159**(1): 34–39.

Gibbs A J and Gower J C. 1960. The use of a multiple-transfer method in plant virus transmission studies: Some statistical points arising in the analysis of results. *Annals of Applied Biology* **48**: 75–83.

Green M R and Sambrook J. 2012. *Molecular Cloning: A Laboratory Manual*, 4th edn, pp. 448. Cold Spring Harbor Laboratory Press, New York.

Jeyaseelan T C, Jeyaseelan E C, De Costa D M and Shaw M W. 2019. Selecting and optimising a reliable DNA extraction method for isolating viral DNA in okra (*Abelmoschus esculentus*). *Vingnanam Journal of Science* **14**(1): 07–14.

Kistler L. 2012. Ancient DNA extraction from plants. *Methods in Molecular Biology* **840**: 71–79.

Kushwaha N, Achuit K, Singh A K, Chattopadhyay B and Chakraborty S. 2010. Recent advances in Geminivirus detection and future perspectives. *Journal of Plant Protection Science* **1**: 1–18.

Lazarowitz S G. 1992. Geminiviruses: Genome structure and gene function. *Critical Reviews in Plant Sciences* **11**: 327–49.

Muniyappa V, Venkatesh H M, Ramappa H K, Kulkarni R S, Zeidan M, Tarba C Y, Ghanim M and Czosnek H. 2000. Tomato leaf curl virus from Bangalore (ToLCV-Ban4): Sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. *Archives of Virology* **145**: 1583–96.

Naresh M, Khan Z A, Kumar R, Kale S P, Patil V M, Rajput J C and Dasgupta I. 2019. Occurrence and variability of begomoviruses associated with bhendi yellow vein mosaic and okra enation leaf curl diseases in south-western India. *Virus Disease* **30**(4): 511–25.

Pirttila M A, Hirsikorpi M, Kamarainen T, Jaakola L and Hohtola A. 2001. DNA isolation methods for medicinal and aromatic plants. *Plant Molecular Biology Reporter* **19**(3): 273.

Sambrook J and Russell D W. 2001. Detection of DNA in agarose gels. *(In) Molecular Cloning, A Laboratory Manual*, 3rd edn, pp. 5–14. Cold Spring Harbor Laboratory Press, New York.

Seth T, Mishra G P, Singh B, Kashyap S, Mishra S K, Tiwari S K and Singh P M. 2018. Optimisation of quality DNA isolation protocol from various mucilage-rich cultivated and wild *Abelmoschus* spp. and its validation through PCR amplification. *Vegetable Science* **45**(1): 1–6.

Singh S J and Dutta O P. 1986. Enation leaf curl of okra- A new virus disease. *Indian Phytopathology* **39**: 328–29.

Stafford C A, Walker G P and Ullman D E. 2012. Hitching a ride: Vector feeding and virus transmission. *Communicative and Integrative Biology* **5**: 43–49.

Statista. 2023. Worldwide Okra Production. https://www.statista.com/outlook/20030000/100/worldokraproduction/worldwide

Venkataravanappa V, Reddy C N L, Jalali S and Reddy M K. 2012. Molecular characterisation of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes* **44**: 522–35. https:// doi.org/ 10. 1007/ s11262- 012-0732-y